### Etiological and Pathological Studies on the Needle Blight of *Cryptomeria japonica*-III

## A comparison between Cercospora cryptomeriae SHIRAI and Cercospora sequoiae Ellis et Everhart

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### Introduction

Cercospora cryptomeriae SHIRAI described by KITAJIMA  $(1916)^{20}$  has been known as the most virulent pathogen causing blight and canker of Cryptomeria japonica D. Don (Itô et al. 1952<sup>11</sup>), 1954<sup>13</sup>), Itô 1953<sup>12</sup>).

Since 1950, a distinctive blight of Sequoia gigantea (LINDL.) DECAISNE [Sequoiadendron giganteum (LINDL.) BUCHHOLZ] has been observed by the present authors in many forestry nurseries in Japan. Symptoms on Sequoia gigantea are very similar to those on Cryptomeria japonica. The causal fungus of the blight of Sequoia was experimentally identified as Cercospora cryptomeriae SHIRAI (ITÔ et al. 1958)<sup>14</sup>).

Judging from the description in literature, *Cercospora cryptomeriae* SHIRAI morphologically closely resembles *Cercospora sequoiae* ELLIS et Ev. (1887)<sup>3)</sup> described in North America as a fungus inhabiting *Sequoia gigantea*, and, accordingly, Irô *et al.* (1958)<sup>14)</sup> suggested that these two fungi might be the same species.

Examinations were made of ELLIS and EVERHART'S type specimen of *Cercospora sequoiae* on *Sequoia gigantea* and other specimens of the same fungus on *Cupressus arizonica* kindly sent by Dr. C. S. HODCES. With cultures of the fungus supplied by him, inoculation experiments were carried out on Japanese conifers.

This paper presents a comparative study between *Cercospora cryptomeriae* SHIRAI in Japan and *C. sequoiae* ELLIS et Ev. in America. A part of the study was preliminarily reported by Itô *et al.*  $(1958)^{14}$  and Itô  $(1965)^{15}$ .

The authors wish to express their sincere appreciation to Dr. C. S. HODGES, of Forestry Sciences Laboratory, Southeastern Forest Experiment Station, U. S. A. for his kindness in sending specimens and cultures of *Cercospora sequoiae* ELLIS et Ev. and an allied species in his country, and for encouragement and advice given during the course of the study. They are also indebted to Mr. Kunihiko SATÔ, of Tohoku Branch, the Government Forest Experiment Station, for his favor in connection with the supply of the experimental materials. Thanks are due to Mr. Tadashi Uozumi, of the Government Forest Experiment Station, for taking electron micrographs, and also to Mr. Michio NAKAGAWA for assistance in the preparation of illustrations.

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# Blight and canker of *Cryptomeria japonica*, and the causal fungus, *Cercospora cryptomeriae* Shirai

### Blight of seedlings

Blight of *Cryptomeria*-seedlings which has been well known among Japanese foresters and plant pathologists under the name of "Aka-gare-byô"\*<sup>1</sup> is the most important disease of Japanese forest trees. In about 1910, the disease was first observed in Ibaraki Prefecture, the central part of Honshu, the main island of Japan, and it spread with such alarming rapidity that by 1921 it was serious in many portions of Honshu, Shikoku and Kyushu (Irô 1965)<sup>16</sup>.

The disease works in both seedling and transplant beds. Moist seasons favor it. The disease usually becomes epidemic, often destroying the entire bed. Seedlings younger than 2 years old are killed in a single season when the disease is epidemic. The appearance of the diseased seedling is like that of seedlings killed by drought (Plates  $1\sim 2$ ).

It is generally said that, unless protective measures are taken, almost all of the seedlings become severely affected by the disease in those nurseries where it is prevalent. The standard practice in combating it is to use copper fungicides in the form of a spray; to date, Bordeaux mixture has proven the most effective and economical. Spraying with chemicals should be combined with sanitary practices for best results. Where the blight is well established a regular spray schedule with the fungicide is essential to production of Cryptomeria-seedlings (Nohara & ZINNO 1952)<sup>24</sup>.

The cause of the disease was first studied by KAWAMURA  $(1913)^{19}$  who attributed it to a new species of the genus *Phyllosticta* (*Phoma*) without any artificial inoculation, and named it *Phyllosticta* (*Phoma*) cryptomeriae KAWAMURA. The same opinion was published by KASAI  $(1914)^{17}$ . Two years later, KITAJIMA  $(1916)^{20}$ , however, described *Cercospora cryptomeriae* SHIRAI as another pathogen of the disease. Since then, several papers have been published on the cause of the blight. Bokura  $(1917)^{11}$  reported that *Pestalotia shiraiana* P. HENNINGS had to be treated as one of the causal fungi of the disease, but he did not indicate any experimental proof. *Macrophoma sugi* HARA was also added by HARA  $(1923)^{6}$  as one of the causes of the disease.

The true nature of the disease was not established until ITÔ *et al.* in 1952<sup>11)</sup>, made complete inoculation experiments with many fungi containing *Phyllosticta* (*Phoma*) cryptomeriae KAWAMURA, *Pestalotia shiraiana* P. HENNINGS and *Macrophoma sugi* HARA, and proved beyond doubt that this disease was mainly caused by *Cercospora cryptomeriae* SHIRAI.

### Canker of adult trees

Sunken cankers on trunks of *Cryptomeria japonica* in plantations were first discovered by K<sub>ITAJIMA</sub> (1927<sup>21)</sup>, 1928<sup>22)</sup>), who named it "Mizo-gusare-byô"\*<sup>2</sup>. He observed clamp connections in the mycelium of a parasite in the rotted wood, and therefore erroneously assumed the cause to be an unknown Basidiomycete. Several years later, KITAJIMA (1933)<sup>23)</sup>, however, expressed no opinion as to the cause of the canker. The true cause of the disease

<sup>\*1</sup> The Japanese word "aka" means red; "gare", blight; and "byo", disease in English, respectively.

<sup>\*2</sup> Japanese word "mizo" means trench-like; "gusare", rot; "byô", disease in English, respectively.

remained unknown for many years until Irô, in 1953<sup>12</sup>, proved that the disease of trunks was initiated from the necrotic lesion on main stems produced by *Cercospora cryptomeriae* SHIRAI in the seedling stage (Plate 2, D).

The fungus that kills needles and branchlets attacks also young stems of seedlings, and makes necrotic lesions or cankers. Main stem cankers on seedlings are usually perennial, and develop to sunken cankers on trunks of adult trees by the active functions of the fungus, year after year. Most cankered trees are infected by wood-decaying fungi entering through the cankers so that even if the trees live to reach merchantable size, the boles will be worthless (Plate 3).

### Cercospora cryptomeriae SHIRAI

Cercospora cryptomeriae SHIRAI was first described by KITAJIMA (1916)<sup>20)</sup> as one of the causal fungi of the blight of Cryptomeria-seedlings as follows:

Cercospora cryptomeriae Shirai in Kitajima, K. (1916). Bot. Mag. (Tokyo) 30, 411~414. semi nomen nudum

Conidiophores dark brown, paler and more narrow toward the tip, sparingly septate, not branched, straight or slightly curved, tip rounded or acute bluntly; conidia olivaceus, helminthoid, slightly curved or rarely straight, very granulous,  $4\sim 6$  septate, base large, tip slender,  $6\sim 7\times 66\sim 70\mu$ .

Host: Seedlings of Cryptomeria japonica D. DON

SAWADA (1928)<sup>28)</sup>, who studied by the material collected in Taiwan (Formosa), indicated in his description of the fungus that the conidia were echinulated.

The following description of this fungus was made by Itô et al. (1952)<sup>11)</sup>:

Fruiting amphigenous; stromata semi-spherical, dark brown, covered densely with fascicles; conidiophores light yellowish brown, straight or slightly curved, not branched,  $1\sim 2$  septate,  $4\sim 6\times 40\sim 80\mu$ ; conidia light yellwish brown to olivaceous, clavate or cylindric, straightly to mildly curved, echinulate,  $3\sim 7$  septate, end rounded, frequently constricted slightly at septum,  $5\sim 9\times 30\sim 85\mu$  (Plate 6, Text-fig. 1).

Inoculation experiments with Cercospora cryptomeriae SHIRAI were made by the authors to the following conifers: Abies veitchii, Larix leptolepis, Picea hondoensis, Pinus bicolor var. acicularis, P. caribaea, P. echinata, P. radiata, P. strobus, P. taeda, P. thunbergii, Cryptomeria japonica, Sequoia sempervirens<sup>\*1</sup>, Chamaecyparis obtusa and Thujopsis dolabrata.

Results showed that all of the tree species tested except *Cryptomeria japonica* could not be infected by the fungus.

### Blight of Sequoia gigantea seedlings and its causal fungus

### Symptoms and damage

The distinguishing symptom of the disease is a browning of the needles on the lowest branchlets adjacent to the stem. Infection gradually progresses upward and outward. Affected needles and branchlets, and lesions on stems turn bronze and necrotic. Later, the diseased seedlings become grayish-brown and are severely defoliated. The symptoms and effects of the disease are so striking that the diseased plants attract attention from a distance. Almost all of the young trees are usually killed by the disease within a few years.

<sup>\*1</sup> Seedlings of Sequoia sempervirens are frequently attacked by Cercospora exosporioides BUBAK, which is quite different from C. cryptomeriae SHIRAI (HASHIMOTO 1959)<sup>7)</sup>.

### Table 1. Dimension of Cercospora cryptomeriae SHIRAI $(\mu)$ .

a. Conidium

Host	Locality and	Length		Width		Number of septum	
	date of collection	Range	Average	Range	Average	Range	Mode
Cryptomeria japonica	Meguro, Tokyo Aug. 11 '49	30~66	47.7	5~6	5.7	3~5	3
do.	Usui, Gunma Aug. 28'49	39~85	57.9	5~9	6.3	2~7	5
Sequoiae gigantea	Seijo, Tokyo Sept. 18'50	33~72	53	3~6	4.5	3~7	5
do.	Kyowa, Akita* July 13 '56	31~66	47	4~7	5.3	3~6	4
do.	Tanashi, Tokyo July 31'57	42~73	56	4~5	4.5	3~7	5

### b. Conidiophore

Host	Locality and date	Le	ngth	Width	
	of collection	Range	Average	Range	Average
Sequoia gigantea	Seijo, Tokyo Sep. 18 '50	24~51	32	4~6	4.4
do.	Kyowa, Akita* July 13'56		36		-
do.	Tanashi, Tokyo July 31 '57	33~66	53		4.2

Note: \* Measured by Mr. K. SATO.

The symptoms of blighted Sequoia-seedlings are very similar to those of the blight of Cryptomeria-seedlings caused by Cercospora cryptomeriae SHIRAI (Plates 1,  $4\sim5$ ).

Nurserymen have been experiencing difficulty in establishing planting of *Sequoia gigantea* in Japan. The difficulty is considered to be due primarily to this blight disease.

### Morphologic characters of the causal fungus

The fungus associated with the blighted *Sequoia*-seedling closely resemble *Cercospora cryptomeriae* SHIRAI in respect to the size, shape and color of the conidia and conidiophores. As shown in Table 1 and Text-fig. 1, the fungus of *Sequoia* is indistinguishable morphologically from *C. cryptomeriae* SHIRAI.

### Macroscopic appearances of the fungous colony

Three mono-conidial isolates from Sequoia gigantea and one mono-conidial isolate from Cryptomeria japonica were cultured on potato-sucrose agar at  $26^{\circ}$ C. Hosts and localities of these isolates are given in Table 2.

Colonies grow slowly, are at first pale yellowish green, and then elevated, dark green, covered with white aerial mycelium in the central part. Macroscopic appearances of the colonies of the fungus isolated from *Sequoia gigantea* are much accordant with those of the fungus isolated from *Cryptomeria*-seedling.

### **Inoculation experiments**

Experiment-1. Isolates from Sequoia gigantea and Cryptomeria japonica were respectively inoculated to potted Cryptomeria-seedlings. As the fungi did not sporulate in culture,

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No. of isolate	Host	Locality
No. 1	Sequoia gigantea	Seijo, Tokyo
No. 2	do.	Tanashi, Tokyo
No. 3*	do.	Kyowa, Akita
No. 4	Cryptomeria japonica	Usui, Gunma

Table 2. Hosts and localities of the isolates tested.

Note: \* Original isolation was made by Mr. K. SATO.

the inoculum was prepared by breaking the cultured mycelium in sterile distilled water, then filtering its fragments through double sheets of cotton cloth. The fungous suspension was sprayed on the 2-year-old seedlings, and bell jars on the pots were kept to maintain a humid atmosphere with absorbent cotton containing water. The jars were removed after 2 days. Then, seedlings inoculated were placed under green-house conditions. Check plants were similarly treated except that they were atomized with water instead of the fungous suspension.

Twenty days after inoculation (June  $19 \sim July 9$ ), brown discoloration, an incipient symptom of the disease, began to appear on the needles, and then blight or necrosis, a typical

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			Resul	t of the expe	eriment
Tree species inoculated	No.	Isolate used	Number of needles inoculated(a)		b/a×100(%)
	1 2 3	Host: Cryptomeria japonica Locality: Usui, Gunma	1,320 1,329 1,128	49 80 92	4 6 8
Cryptomeria japonica	4 5 6	Host: Sequoia gigantea Locality: Seijo, Tokyo	1,488 1,696 1,639	260 140 206	18 8 13
	7 8 9	Check	1,084 1,435 1,153	0 0 0	0 0 0
	1 2 3	Host: Cryptomeria japonica Locality: Usui, Gunma	490 788 575	175 354 169	36 45 29
Sequoia gigantea	4 5 6	Host: Sequoia gigantea Locality: Seijo, Tokyo	1,279 1,714 670	816 1,013 390	64 59 58
	7 8 9	Check	1,249 748 809	0 0 0	0 0 0

## Table 3. Cross inoculations with Cercospora cryptomeriae SHIRAI to Cryptomeria japonica and Sequoia gigantea.

symptom, was observed at the end of 1 month's experiment. Conidiophores and conidia typical of the *Cercospora* were formed on blighted needles and stems resulting from the inoculation, whereas all the check seedlings remained healthy.

The symptoms produced by inoculation were typical of the disease as it occurred naturally. There were no difference in pathogenicity between the isolate from *Sequoia* and that from *Cryptomeria* (Plate 5, C).

Experiment-2. Cross inoculations with the isolate from *Cryptomeria* and that from *Sequoia* were performed by the same method as in the previous experiment to *Cryptomeria* and *Sequoia*-seedlings.

Results obtained at the end of the experiments (September 15 $\sim$ November 20) are summarized in Table 3.

As shown in Table 3, these two isolates are equally pathogenic to *Cryptomeria japonica* and *Sequoia gigantea*, and cause severe blight in both tree species. It will be seen from Table 3 that the isolate from *Sequoia* is more virulent than that from *Cryptomeria*.

The fungus causing blight of *Sequoia gigantea* in Japan is quite accordant with *Cercospora* cryptomeriae SHIRAI in morphologic characters, appearances of the mycelial colony and pathogenicity as mentioned above.

### Cercospora cryptomeriae Shirai and C. gigantea Ellis et Ev.

### Description of Cercospora sequoiae<sup>\*1</sup> ELLIS et Ev.

The fungus was first described by ELLIS and EVERHART (1887)<sup>3)</sup> from foliage of Sequoia gigantea collected at Germantown Nurseries, Pennsylvania, U. S. A. The original descrip-

<sup>\*1</sup> In SACCARDO'S (1892)<sup>27)</sup> Sylloge fungorum, the species name was spelled as sequoijae.

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tion was as follows: Forming large, compact, olivaceous tufts which, under the lens, resemble the perithecia of a *Sphaerella*; hyphae (under the microscope) ferruginous, brown, abruptly bent, subnodulose and toothed, sparingly septate,  $50 \sim 70 \times 4 \sim 5\mu$ , [conidia] oblong, becoming obclavate, same color as the hyphae,  $40 \sim 70 \times 4 \sim 6\mu$ ,  $3 \sim 5$  septate and some of

Host	Conidium	Conidiophore
Sequoia gigantea*	$37 \sim 53 \times 5 \sim 7\mu$ , pale brown to olivish-brown, echinulate	$55 \sim 70 \times 5 \sim 8\mu$ , pale brown to olivish-brown, smooth in most parts, but echinulate at the tip, usually aseptate
Cupressus arizonica**	$42\sim63\times5\sim7\mu$ , pale brown to olivish-brown, echinulate	$27 \sim 78 \times 4 \sim 5\mu$ , brown to olivish-brown, echinulate at the tip, usually aseptate

Table 4. Dimension of *Cercospora sequoiae* ELLIS et Ev. measured by the present authors.

Notes: \* Type specimen; \*\* collected in U.S.A. Both specimens were sent by Dr. C.S. Hobges.









Text-fig. 3. Cercospora sequoiae ELLIS et Ev. on Cupressus arizonica collected in U.S.A. ('--' = $10\mu$ ) Text-fig. 4. Cercospora sequoiae ELLIS et Ev. var. juniperi ELLIS et Ev. on Juniperus virginiana collected in U. S. A.  $(, - = 10\mu)$  them strongly constricted at the septa.

The feature that conidia of this fungus are echinulate was first found by  $CHUPP(1953)^{23}$ and again pointed out by Hodges  $(1962)^{83}$  who examined the type specimen, though ELLIS and EVERHART (1887)<sup>23</sup> apparently failed to note the echinulation, for they made no mention of it in their description.

The morphology of *Cercospora cryptomeria* SHIRAI agrees well with the description for *Cercospora sequoiae* ELLIS et Ev. And, therefore, ITô *et al.* (1958)<sup>14</sup>) reported that the blight and canker fungus of *Cryptomeria* under the name of *Cercospora cryptomeriae* SHIRAI in Japan might be identical with *Cercospora sequoiae* ELLIS et Ev., so far as could be judged from the literature.

# Comparison between *Cercospora cryptomeriae* SHIRAI and *C. sequoiae* ELLIS et Ev. in morphology

With the materials collected both in North America and Japan, a direct comparison between these two fungi was made by the present authors.

As shown in Text-figs.  $1\sim3$  and Tables 1, 4 there are no differences in morphologic characteristics between *Cercospora cryptomeriae* SHIRAI and *C. sequoiae* ELLIS et Ev.

# Mycelial growth of Cercospora cryptomeriae SHIRAI and C. sequoiae ELLIS et Ev. in culture media

One hundred cc. of each of the 5 kinds of the solution, viz., potato sucrose solution, malt decoction, CZAPEK'S solution, Cryptomeria-needle decoction and SAITO'S soy solution were poured into 200 cc. Erlenmyer flasks, and each solution was set up in quinplicate. After sterilization they were inoculated with the fungous isolates, and were kept at about  $25^{\circ}$ C. At the end of 45 days' culture, the mycelial colonies were thoroughly washed with distilled water and dried in the oven. The dry weight of the mycelium was measured and averaged for 5 flasks. The fungous isolates used and the results of the experiment are presented in Table 5.

As shown in Table 5, there are no remarkable differences in mycelial growth among the isolates of *Cercospora cryptomeriae* SHIRAI and *C. sequoiae* ELLIS et Ev. On the contrary, the mycelium of *Cercospora sequoiae* ELLIS et Ev. var. *juniperi* ELLIS et Ev. grows very sparsely in all of the culture media tested (Plate 7).

Macroscopic appearances of the mycelial colonies of *Cercospora sequoiae* ELLIS et Ev. are very similar to those of *C. cryptomeriae* SHIRAI. The culture of *Cercospora sequoiae* ELLIS et Ev. var. *juniperi* ELLIS et Ev. is distinctly different from those of these fungi.

### Inoculations with Cercospora sequoiae ELLIS et Ev. to some conifers

In 1963 and 1964, repeated inoculations with Cercospora sequoiae ELLIS et Ev. and C. cryptomeriae SHIRAI were carried out by the same procedures as in the previous experiments to the following tree species: Cryptomeria japonica, Chamaecyparis obtusa, Ch. pisifera, Cupressus sempervirens, and Thuja occidentalis. The fungous isolates used, tree species inoculated and the results obtained are summarized in Tables  $6\sim7$ .

From the results shown in Tables  $6\sim7$ , it is clear that Cercospora cryptomeriae SHIRAI is pathogenic to only Cryptomeria japonica, while C. sequoiae ELLIS et Ev. affects Cryptomeria japonica, Chamaecyparis pisifera and Ch. obtusa, so far as the tree species tested in these experiments are concerned. Here, it many be said that host range of Cercospora sequoiae ELLIS et Ev. is larger than that of C. cryptomeriae SHIRAI.

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			Cu	ilture med	lium	
Fungus	Isolate	Potato sucrose sol.	Malt decoct.	Czapek's sol.	Cryptomeria needle decoct.	Saito's soy sol.
Cercospora cryptomeriae Shirai	Host: Cryptomeria japonica Locality: Morioka, Iwate*	0.61	0.97	0.12	0.16	1.29
do.	Host: Sequoia gigantea Locality: Kyowa, Akita*	0.60	1.17	0.18	0.11	1.12
Cercospora sequoiae Ellis et Ev.	Host: Chamaecyparis pisifera Locality: North Carolina, U.S.A.**	0.54	1.43	0.17	0.16	0.93
do.	Host: Cupressus arizonica Locality: South Carolina, U.S.A.**	0.60	0.60	0.22	0.12	1.44
do.	Host: Cupressus arizonica Locality: Louisiana, U.S.A. **	0.35	0.53	0.15	0.13	1.80
Cercospora sequoiae Ellis et Ev. var. juniperi Ell. et Ev.	Host: Cupressus arizonica Locality: Georgia, U. S. A. **	0.14	0.38	0.14	0.11	0.36

Table 5.	Mycelial growth of Cercospora cryptomeriae SHIRAI, C. sequoiae Ellis et Ev.,
	and C. sequoiae Ellis et Ev. var. juniperi Ellis et Ev. (g)

Notes: \* Isolated by Mr. K. SATO. \*\* Cultures were obtained from Dr. C. S. HODGES. Table 6. Inoculation experiments with *Cercospora sequoiae* Ell. et Ev. to several conifers. Experiment-1 (July 26, 1963 $\sim$ ).

Isolate used	Tree species inoculated	Symptom
Host: Chamaecyparis pisifera Locality: Releigh, North Carolina, U.S.A.*	Cryptomeria japonica Chamaecyparis obtusa	+ _
Host: Cupressus arizonica Locality: St. George, South Carolina, U.S.A.*	Cryptomeria japonica Chamaecyparis obtusa	+ -

Experiment-2 (August 13, 1963 $\sim$  ).

Host: Chamaecyparis pisifera Locality: Releigh, North Carolina, U.S.A.*	Cryptomeria japonica Chamaecyparis pisifera Cupressus sempervirens Thuja occidentalis	+
Host: Cupressus arizonica Locality: St. George, South Carolina, U.S.A.*	Cryptomeria japonica Chamaecyparis pisifera Cupressus sempervirens Thuja occidentalis	+

Note: \* Cultures were obtained from Dr. C. S. Hodges.

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# Table 7. Inoculation experiments with *Cercospora cryptomeriae* SHIRAI and *C. sequoiae* ELLIS et Ev. to three Japanese conifers.

Experiment-1 (June 11, 1964 $\sim$  )

		Tree species	Result of the experiment		
Fungus	Isolate used	inoculated	Symptom	Conidium formation on lesion	
	Host: Cryptomeria japonica	Cryptomeria japonica	+	+	
<b>a</b> .	Locality: Morioka, Iwate*	Chamaecyparis pisifera	—	-	
Cercospora cryptomeriae		Chamaecyparis obtusa		—	
Shirai	Host: Sequoia gigantea	Cryptomeria japonica	+	+	
		Chamaecyparis pisifera	—		
		Chamaecyparis obtusa			
	Host: Cupressus arizonica	Cryptomeria japonica	+	+	
	Locality: Bogalusa,	Chamaecyparis pisifera	+	+	
	Louisiana, Ú.S.A.**	Chamaecyparis obtusa	—	_	
Cercospora	Host: Chamaecyparis pisifera	Cryptomeria japonica	+	+	
sequoiae Ell.	Locality: Releigh, North	Chamaecyparis pisifera	+	+	
et Ev.	Carolina, U.S.A.**	Chamaecyparis obtusa	—	-	
	Host: Cupressus arizonica**	Cryptomeria japonica	+	+	
	Locality: St. George, South	Chamaecyparis pisifera	+	+	
	Carolina, U.S.A.**	Chamaecyparis obtusa	-	-	

### Experiment-2 (July 22, 1964 $\sim$ )

	Host: Cryptomeria japonica Locality: Morioka, Iwate*	Cryptomeria japonica Chamaecyparis pisifera	+	+
Cercospora cryptomeriae	Locality. Morioka, Twate	Chamaecyparis obtusa		
Shirai	Host: Sequoia gigantea	Cryptomeria japonica	+	+
	Locality: Kyowa, Akita*	Chamaecyparis pisifera	-	—
	· · · · · · · · · · · · · · · · · · ·	Chamaecyparis obtusa	-	—
	Host: Cupressus arizonica	Cryptomeria japonica	+	+
	Louisiana IISA **	Chamaecyparis pisifera	+	+
		Chmaecyparis obtusa	_	—
Cercospora	Host: Chamaecyparis pisifera	Cryptomeria japonica	+	+
sequoiae	Locality: Releigh, North	Chamaecyparis pisifera	+	+
Ell. et Ev.	Carolina, U.S.A.**	Chamaecyparis obtusa	-	_
	Host: Cupressus arizonica	Cryptomeria japonica	+	+
	Locality: St. George,	Chamaecyparis pisifera	+	+
	South Carolina, U.S.A.**	Chamaecyparis obtusa	+	+

Notes: \* Isolated by Mr. K. SATO. \*\* Cultures were obtained from Dr. C.S. Hodges.

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Text-fig. 5. Cercospora sequoiae ELLIS et Ev., which was originally isolated from Cupressus arizonica in U.S.A. on Cryptomeria japonica inoculated artificially in Japan.  $(--) = 10\mu$ 



Text-fig. 6. Cercospora sequoiae ELLIS et Ev., which was originally isolated from Cupressus arizonica in U.S.A. on Chamaecyparis obtusa inoculated artificially in Japan.  $(i \rightarrow = 10\mu)$ 

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## Table 8. Dimension of *Cercospora sequoiae* ELLIS et Ev. on three Japanese conifers inoculated artificially $(\mu)$ .

### a. Conidium

Isolate used	Tree species	Length		Width		Number of septum	
isolate used	inoculated	Range	Average	Range	Average	Range	Mođe
Host: Chamaecyparis pisifera Locality: North Carolina, U. S. A. *	Chamaecyparis pisifera	46~73	60.8	5~8	6.1	3~9	5
Host: Cupressus arizonica Locality: South	Chamaecyparis pisifera Chamaecyparia obtusa	$54 \sim 83$ $36 \sim 81$		5~7 5~6	5.6 5.2	$4 \sim 7$ 2~5	5
Carolina, U.S.A.*	Cryptomeria japonica	$44 \sim 84$		5~6	5.2	3~6	5

### b. Conidiophore

Isolate used	Tree species inoculated	Length		Width	
		Range	Average	Range	Average
Host: Chamaecyparis pisifera Locality: North Carolina, U. S. A. *	Chamaecyparis pisifera	49~101	71.5	-	5.2
Host: Cupressus arizonica Locality: South Carolina, U. S. A. *	Chamaecyparis pisifera	46~96	68.4		5.2
	Chamaecyparis obtusa	41~88	60.3	—	5.2
	Cryptomeria japonica	59~101	74.5	4.7~5.2	4.9

Note: \* Cultures were obtained from Dr. C.S. Hodges.

Fruit-bodies of the fungi formed on the inoculated trees are presented in Text-figs.  $5\sim$  6 and Table 8.

### Consideration and conclusion

The most important fungus causing blight and canker of *Cryptomeria japonica* has been known by the name of *Cercospora cryptomeria* SHIRAI in Japan. CHUPP (1953<sup>2)</sup>, p. 439), in his monographic study, commented that "I have not seen this specimen [*C. cryptomeriae* SHIRAI]. The width of the conidiophores and conidia might indicate that it were of some other genus".

From the morphologic characteristics and nature pathogenic to Sequoia gigantea, Irô et al. (1958)<sup>14)</sup> reported that Cercospora cryptomeriae SHIRAI described in Japan in 1916 was indistinguishable from C. sequoiae ELLIS et Ev. from North America, so far as judging from literature can be done.

Recently, HODGES (1962)<sup>8)</sup>, in his comparative study of Cercosporae from some conifers, treated Cercospora thujina PLAKIDAS (1945)<sup>25)</sup> as a synonym of Cercospora sequoiae ELLIS et Ev., and, as hosts of this fungus, he noted the following tree species: Sequoia gigantea, Thuja orientalis, Cupressus arizonica, C. lustica, C. macrocarpa, C. sempervirens, Juniperus virginiana, and Chamaecyparis pisifera.

By a direct comparison of both Japanese and American materials it was revealed that *Cercospora cryptomeriae* SHIRAI was morphologically very accordant with *C. sequoiae* ELLIS et Ev. In mycelial colonies in several culture media there were no remarkable differences between these two fungi.

Inoculations with the culture of *Cercospora sequoiae* ELLIS et Ev. from America showed that the fungus caused blight of *Cryptomeria japonica*, and the symptoms were closely similar to those resulting from *Cercospora cryptomeriae* SHIRAI. Though results of the inoculation experiments conducted in Japan showed that the host range of *Cercospora cryptomeriae* SHIRAI was somewhat smaller than that of *C. sequoiae* ELLIS et Ev., the present authors can not give this as a reason for separating the two fungi.

The foregoing facts lead to the conclusion that *Cercospora cryptomeriae* SHIRAI is synonymous with *C. sequoiae* ELLIS et Ev. as follows:

Cercospora sequoiae Ellis et Everhart (1887)<sup>8)</sup>

syn. Cercospora thujina Plakidas (1945)<sup>25)</sup>

syn. Cercospora cryptomeriae Shirai in Kitajima (1916)<sup>20)</sup>

Some confusion has appeared in literature concerning the placement of this fungus in the genus *Cercospora* because of its echinulate conidia. CHUPP (1953<sup>2)</sup>, p. 440) described that this fungus was not a species of *Cercospora*, and should be placed in the genus *Heterosporium*.

According to the Saccardian system (SACCARDO 1886)<sup>26)</sup> characteristics of cach of the genera *Cercospora* and *Heterosporium* are as follows:

Cercospora Hyphae molliusculae, saepius biophilae; conidia vermicularia, levia.

Heterosporium Hyphae molliusculae, phyllo-caulogenae; conidia oblonga, echinulata.

While this fungus is more similar to *Cercospora* in shape of conidia, it is closer to *Heterosporium* in echinulation in conidia, and is said to be intermediate between these two genera (Irô *et al.* 1958)<sup>14</sup>), though HUGHES (1958)<sup>10</sup>) treated *Heterosporium* KLOTZ (1877) as the same genus as *Cladosporium* LINK (1815).

Under a new system of classification proposed by Hughes (1953)<sup>9)</sup> for the Hyphomycetes based on the method of conidial origin, this fungus was placed in the genus *Cercospora* by HODGES (1962)<sup>8)</sup>.

Echinulation in conidia is an extraordinary character in the member belonging to the genus *Cercospora* (CHUPP 1953<sup>2)</sup>, KATSUKI 1965<sup>18)</sup>), and, therefore, taxonomic position of this fungus will be discussed again in future. For the present, the authors follow Hodges' (1962)<sup>8)</sup> opinion that "it is believed best to leave *Cercospora sequoiae* ..... in *Cercospora* until the limits of the various genera are settled more satisfactorily".

Whether the fungus is indigenous to Japan or was introduced from other countries is an interesting and important problem from the standpoint of epidemiology.

As early as the eleventh century, *Cryptomeria japonica* was cultivated from seeds in some parts of Japan. According to ancient manuscripts in the Tohoku district, the northeastern part of the Japanese main island (Honshu), it is reliably reported that a great number of *Cryptomeria*-seedlings have been cultured in many nurseries since the feudal age, the sixteenth century (ENDÓ 1938)<sup>4</sup>. In the old literature, no records on the trouble suggesting this disease have been found so far as the authors are aware.

Prior to the first description of "Cercospora cryptomeriae SHIRAI" in 1916 (KITAJIMA 1916)<sup>20)</sup>, a blight of Cryptomeria-seedlings caused probably by this fungus occurred suddenly in a part of the main island of Japan in about 1910. Since that time, the disease spread very rapidly and, within a few years it was widely distributed throughout almost all of the districts where cultivated. In Taiwan (Formosa), the fungus which had been introduced probably from Japan, accompanying *Cryptomeria*-seedlings, was first listed by FUJIKURO<sup>5</sup> in 1918.

The fungus has never been found on *Cryptomeria japonica* in natural forests, and, here, the hypothesis tuat the source of its propagation originated from natural forests must be denied. Among native conifers *Cryptomeria japonica* is only one host of the fungus strain distributed in Japan.

Incidentally, up to 1910 the Japanese Government had no legal authority to exclude diseased propagating stocks, and it was only after the quarantine regulation of 1914 that the policy of free entry of diseased plants really ended.

Such being the case, Irô *et al.* (1958)<sup>14)</sup> proposed preliminarily an opinion that the fungus had been probably introduced from North America, accompanying seedlings or stocks of American conifers, especially *Sequoia gigantea*, at about the beginning of this century. This opinion was supported by the late Mr. Kanesuke HARA<sup>\*1</sup>, who had studied forest pathology and mycology in Japan since the beginning of this century.

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<sup>\*1</sup> Personal communication to K. Itô from K. HARA in his lifetime.

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### **Explanation of plates**

### Plate 1

- A. Two-year-old seedlings of Cryptomeria japonica attacked by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.)
- B. Cryptomeria-seedling attacked by "Cercospora cryptomeriae Shirai" (=C. sequoiae Ellis et Ev.)
- C. Seedlings of Sequoia gigantea attacked by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.)
- D. Sequoia-seedling attacked by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae Ellis et Ev.) Plate 2
- A. One-year-old seedling attacked severely by "Cercospora cryptomeriae Shirai" (=C. sequoiae Ellis et Ev.)  $\times 1$

- B. Blight of 3-year-old seedling of Cryptomeria japonica caused by "Cercospora cryptomeriae  $S_{HIRAI}$ " (=C. sequoiae ELLIS et Ev.)  $\times 1$
- C. Needles of Cryptomeria japonica attacked by "Cercospora cryptomeriae Shirai" (=C. sequoiae Ellis et Ev.)  $\times 5$
- D. Necrotic lesion on main stem of Cryptomeria-seedling caused by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae Ellis et Ev.) ×1

Plate 3

- A. Necrotic lesions on green stem of 7-year-old Cryptomeria caused by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.) ×0.8
- B. Cankers on trunk of 5-year-old Cryptomeria caused by "Cercospora cryptomeriae Shirai" (=C. sequoiae ELLIS et Ev.)  $\times 0.5$
- C. Cankers on trunks of about 17-year-old Cryptomeria caused by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.)
- D. Cross section of cankered trunk of Cryptomeria caused by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.)

Plate 4

- A. Two-year-old seedlings of Sequoia gigantea attacked severely by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.) in nursery
- B. Seedlings of Sequoia gigantea attacked by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.) × 0.8
- C. Blight of Sequoia-seedling caused by "Cercospora cryptomeriae Shirai" (=C. sequoiae Ellis et Ev.)  $\times 1$

Plate 5

- A. Blighted needls and necrotic lesions on stem of Sequoia-seedling caused by "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.) ×1.7
- B. Needles of Sequoia-seedling attacked by "Cercospora cryptomeriae Shirai" (=C. sequoiae Ellis et Ev.) ×2.3
- C. Results of the inoculation experiment with "Cercospora cryptomeriae SHIRAI" (=C. sequoiae Ellis et Ev.) to Cryptomeria-seedlings.

From left to right: Check; with isolate from Sequoia gigantea; with isolate from Cryptomeria japonica

Plate 6

Electron micrographs of conidia of "Cercospora cryptomeriae SHIRAI" (=C. sequoiae Ellis et Ev.) on needles of Cryptomeria-seedling. Showing echinulation in conidia.  $A\sim C$ ,  $\times 2,000$ ; D,  $\times 5,000$ 

### Plate 7

Results of culture of "Cercospora cryptomeriae SHIRAI" (=C. sequoiae ELLIS et Ev.), C. sequoiae ELLIS et Ev., and C. sequoiae ELLIS et Ev. var. juniperi ELLIS et Ev. in several culture media

In each series, isolates tested are, from left to right, as follows: Isolate from Sequoia gigantea, Japan; C. sequoiae var. juniperi, U. S. A.; isolate from Cupressus arizonica, Louisiana, U. S. A.; isolate from Chamaecyparis pisifera, U. S. A., isolate from Cupressus arizonica, South Carolina, U. S. A.; isolate from Cryptomeria japonica, Japan.

A, Potato sucrose solution; B, Malt decoction; C, CZAPEK'S solution; D, SAITO'S soy solution

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スギの赤枯病に関する病原学的ならびに病理学的研究 (Ⅲ)

## 病原菌 Cercospora cryptomeriae SHIRAI と北米産 Cercospora sequoiae ELLIS et EVERHART との比較

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### (摘 要)

スギ苗の赤枯病は 1910 年ごろ, 茨城県の一角に突如として発生, またたく間に広く蔓延し, 1921 年 ごろには本州, 四国および九州の各地に修害を与え, 爾来本病はわが国における最も重要な森林病害にあ げられていることは周知のとおりである。

本病の病原菌を最初に研究した川村(1913) は、これを新種と認めて Phyllosticta (Phoma) cryptomeriae KAWAMURA と命名した。翌年笠井 (1914) はその報文においてこの病原菌について 川村の意見を支持し ている。さらに2年後、北島(1916)は本病の病原菌として Phyllosticta (Phoma) cryptomeriae KAWAMURA のほかに Cercospora cryptomeriae SHIRAI をあげて新種の記載を行なった。その後卜蔵 (1917) は Pestalotia shiraiana P. HENNINGS を、または原 (1923) は Macrophoma sugi HARA をそれぞれあげて本病病 原菌に数えるべきだとした。このように、本病の病原菌は諸説紛々として帰一するところなく長年月を経 過したのであるが、伊藤ら (1952) の研究によって、本病を基因する主要な病原菌は Cercospora cryptomeriae SHIRAI にほかならず、他の菌類の病原性はいずれもきわめて徴弱で、実際上ほとんど問題にする 必要がないことが明らかにされ、本病病原に関する長い間の論争にようやく終止符が打たれた。

造林木の幹に縦溝を形成して材質を低下させる溝腐病は北島(1927, 1928)によって発見・命名されたものであるが、この病因はその後長い間不明とされてきた。近年伊藤(1953)によって本病は苗木時代に罹病した赤枯病の茎における胴枯型病斑が、年を経て病状のまったく異なる溝腐病になる経過が追跡されて赤枯病はひとり苗木時代における重要病害であるばかりでなく、その影響は長く林木の時代にまで及ぼすことが明らかにされ病原菌 Cercospora cryptomeriae ShirAI のスギに対する重要性はいっそう強調された。

ところで、分類学上スギにきわめて近縁で北米合衆国原産のギガント・セコイア(Sequoia gigantea)は 由来わが国では生育しないものとされていた。1950年以来、各地の苗畑に播種されたギカント・セコイア 苗はある種の疾病によってはなはだしく侵され、発芽後数年にして全滅する惨状が目撃され、その病徴は スギの赤枯病に酷似することが観察された。そしてこのような罹病苗には Cercospora 菌が例外なしに認 められ、形態比較および交互接種試験によってギガント・セコイアの菌はスギの赤枯病菌 Cercospora cryptomeriae Shirkai にほかならぬことが伊藤ら (1958)によって立証された。

北米合衆国においてガギント・セコイアに記載されたものに Cercospora sequoiae ELLIS et EVERHART (1887) という菌がある。スギの赤枯病菌 Cercospora cryptomeriae SHIRAI は文献の記載文と比較するか ぎり、C. sequoiae ELLIS et Ev. にきわめて近似で両者間に区別点が見い出されない。それに、C. cryptomeriae SHIRAI はわが国においてギガント・セコイアをも侵すことから、この菌は C. sequoiae ELLIS et

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Ev. と同一のものではあるまいかと伊藤ら(1958)によって報じられた。

最近にいたり幸にも北米合衆国の C.S. HODGES 博士の好意によって ELLIS・EVERHART のタイプ標本 を含む C. sequoiae ELLIS et Ev. の多数の標本および培養を入手することができて, これとスギ赤枯病 菌 C. cryptomeriae SHIRAI を直接比較検討する機会がえられた。形態, 培地上の菌叢の状況および接種試 験による病原性を比較した結果, これら両菌はいずれの性質においてもよく一致し, 同一菌として取りあ つかうべきであるとの結論に到達, 長い間の疑問がようやく 氷解する 運びに なった。C. sequoiae ELLIS et Ev. (1887) の記載発表は北島 (1916) による C. cryptomeriae SHIRAI のそれに先だつこと 29 年, し たがってスギの赤枯病菌の学名としては C. sequoiae ELLIS et Ev. をあてる のが 至 当 で, C. cryptomeriae SHIRAI はその異名とすべきものである。

なお、スギの赤枯病菌の分生胞子には細疣あるいは細棘(echinulation)があり、これは他の Cercospora 属菌類にはまったくみられない特徴である。それで伊藤ら(1958)はすでに、本菌は Cercospora 属と Heterosporium 属の中間的性質を有する菌であると指摘した。不完全菌類の分類基準は今日なお明確にさ れていないが、HUGHES(1953)の提唱した分生子梗の性質および分生胞子の形成様式による分類基準にし たがって、ここでは本菌を Cercospora 属に止めておくが、この点に関してはさらに後日の検討にまつこ とにしたい。

スギの赤枯病菌 Cercospora sequoiae ELLIS et Ev. (=C. cryptomeriae SHIRAI) はそもそもわが国原 産のものか,あるいはまた外国から輸入されたものであるかは疫学(epidemiology)上興味ある重要な問 題である。記録によるとわが国で播種によってスギ苗を育てたのは早くも第11世紀にさかのぼるという。 これほど古い時代のことは別にしても東北地方では第16世紀以後藩政時代に播種によるスギ苗の養成が 多量に行なわれた。藩政時代の記録をひととおりあたってみたが、本病の被害と考えられる記事はまった く見い出されない。防除薬剤のない古い時代に本病がひとたび発生すればスギ苗はほとんど全滅の惨害を 受けたはずで、これをはっきりと病気だとは気づかない当時の人々でも、何かの形で記録に止めておいた であろうと思われるが、本病の被害と推察される記述は見られない。また、本病の病原菌がスギ天然生木 から苗畑に移ったと判断される形跡もまったくない。

わが国で植物検疫に関する法律が発布されたのは 1914 年で,それ以前は諸外国から苗木等の輸入が自 由に行なわれたらしく,このことは古い時代に開設された苗畑の周辺に外国種の老木が残存していること から容易に推察される。スギの赤枯病がわが国で最初に問題になったのは 1910 年ごろのことであるから, それ以前には当然外国から各種の苗木がわが国に持ちこまれたにちがいない。

以上のことがらからみて, Cercospora sequoiae ELLIS et Ev. (=C. cryptomeriae SHIRAI) は北米合衆 国から輸入された針葉樹苗, おそらくギガント・セコイア苗に潜在してわが国に侵入, これからスギ苗に 移って赤枯病病原菌になったと推理される理由がある。もっとも, 今日となってはこれを具体的に証明す ることはほとんどまったく不可能で, 想像の域を一歩も出ないのであるが, 伊藤ら (1958) のこの大胆な 推理について,本世紀初頭から樹病研究に従事し, 自ら山林経営も行なった故原 摂祐氏の賛意をかつて えたことを記しておく。 スギの赤枯病に関する病原学的ならびに病理学的研究(III)(伊藤ほか) — Plate 1 —















— Plate 2 —



スギの赤枯病に関する病原学的ならびに病理学的研究(π)(伊藤ほか) — Plate 3 —









スギの赤枯病に関する病原学的ならびに病理学的研究(Ⅲ)(伊藤ほか) — Plate 5 —



— Plate 6 —

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スギの赤枯病に関する病原学的ならびに病理学的研究(Ⅲ)(伊藤ほか) — Plate 7 —